bio-protocol Preprint

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Passaging of organoids

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Updated date: Sep 28, 2021

An abbreviated version of this protocol was published in eLIFE in Jun 2017

Human embryonic lung epithelial tips are multipotent progenitors that can be expanded in vitro as long-term self-renewing organoids

DOI: 10.7554/eLife.26575

Detailed protocol

Splitting and maintenance of human embryonic lung organoids

Before you start

- Thaw matrigel on ice.
- Pre-warm plates by putting them into the incubator.
- Prepare sufficient quantities of ice-cold wash medium.

Things you will need

48-well tissue culture plates (Greiner Bio One cat.no. 677980)
15ml falcon tubes
Pipettes and filter tips (p1000, p200, p20)
Matrigel, Basement Membrane Matrix, Growth Factor Reduced (GFR), Phenol Red-free (BD cat.no. 356231)
Cold Advanced DMEM/F12 (ThermoFisher Scientific 12634010) for washing
Human embryonic lung self-renewal growth medium (protocol 4.2)

Splitting the organoids

1. Pipette 1ml cold Advanced DMEM/F12 into the well containing the organoids to break the basement membrane (matrigel) bubble.
2. Aspirate the matrigel and organoids into the 1ml pipette and transfer to a 15 ml falcon tube.
3. Add approximately 10 ml of cold Advanced DMEM/F12 into the falcon tube and invert 2-3 times.

Washing in cold medium removes the old matrigel.

1. Spin 200-500 rcf, 5 minutes at room temperature. Aspirate media leaving about 1 ml.
2. Repeat the wash (steps 3 and 4) and place the tube containing organoids on ice.
3. Using a P200 pipette, manually break up the organoids by pipetting up and down approximately 20 times.
4. Add approximately 10 ml of cold Advanced DMEM/F12 into the falcon tube.
5. Spin 200-500 rcf, 5 minutes at room temperature, aspirate as much medium as possible and transfer the organoids to ice.
6. Resuspend the pellet in 30 – 150µl matrigel and pipette up and down to mix.

Typically we add 30µl matrigel per well of 48 well plate (ie resuspend the pellet in 150µl matrigel if splitting organoids at a 1:5 ratio).

1. Seed into a pre-warmed 48 well plate (30µl matrigel/organoid mix per well). Pipette slowly to avoid bubbles and seed organoids in the centre of the well to generate a bubble/dome of matrix.
2. Allow to solidify in the incubator for 10 – 15 minutes for the basement membrane mix to polymerize.
3. Overlay with 300µl self-renewing medium per well.

Note: organoids can also be grown in 24 well plates using 50µl matrigel per well and 600µl self-renewing medium.

For routine maintenance replace the culture medium every 3-4 days.

Media protocols

Advanced DMEM/F12 +++ medium

Store at 4 ˚C for 4 weeks	
Advanced DMEM/F12	500 ml
Glutamax 100x	5 ml
Hepes 1M	5 ml
PenStrep 100x	5 ml

Human embryonic lung self-renewing medium, 20ml

Store at 4 ˚C for 4 weeks	
Advanced DMEM/F12 +++	17.4 ml
B27 supplement (50x)	400 µl
N2 supplement (100x)	200 µl
n-Acetylcysteine (500 mM)	50 µl
mouse EGF (500 µg/ml)	10 µl
mouse Noggin (100 µg/ml)	20 µl
R-Spondin conditioned medium	1 ml

FGF10 (100 µg/ml)	20 µl
FGF7 (50 µg/ml)	40 µl
CHIR99021 (10 mM)	6 µl
SB43152 (10 mM)	20 µl

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Rawlins, E. (2021). Passaging of organoids. Bio-protocol Preprint. bio-protocol.org/prep1384.
2. Nikolić, M. Z., Caritg, O., Jeng, Q., Johnson, J., Sun, D., Howell, K. J., Brady, J. L., Laresgoiti, U., Allen, G., Butler, R., Zilbauer, M., Giangreco, A. and Rawlins, E. L.(2017). Human embryonic lung epithelial tips are multipotent progenitors that can be expanded in vitro as long-term self-renewing organoids. eLIFE. DOI: 10.7554/eLife.26575